

## A MASS CULTURE METHOD FOR *Tetraselmis* sp. - A PROMISING FOOD FOR LARVAL CRUSTACEANS<sup>1</sup>

G. W. Griffith, M. A. Murphy Kenslow and L. A. Ross  
National Marine Fisheries Service Gulf Coastal Fisheries Center  
Galveston Laboratory  
Galveston, Texas 77550

### ABSTRACT

*Tetraselmis* sp., a green flagellate, shows promise as a good source for larval crustaceans. This paper discusses culture techniques. Typical densities produced in the mass-culture tanks ranged from 275,000 to 450,000 cells per ml.

### INTRODUCTION

Phytoplankton is a necessary part of the diet of many aquatic organisms. Many crustaceans, including penaeids, require phytoplankton as food during their early larval stages (Hudinaga, 1942; Cook and Murphy, 1966, and Mock and Murphy, 1970). Consequently, in any attempt to rear penaeid shrimp from eggs, arrangements must be made to assure a plentiful supply of algal food during the protozoal stage of development.

Hudinaga (1942) in his early work used the diatom *Skeletonema costatum* which he considered to be an excellent food for larval shrimp. Cook and Murphy (1966) and later Mock and Murphy (1970) used *Skeletonema* sp. to feed shrimp larvae at the Galveston Laboratory. They also used other algal organisms as food, comparing these with *Skeletonema*.

<sup>1</sup>Contribution No. 354, National Marine Fisheries Service Gulf Coastal Fisheries Center, Galveston Laboratory, Galveston, Texas 77550.

In 1965 we isolated a green flagellate from water samples collected from East Lagoon, Galveston. This organism was maintained in the laboratory until 1971 when we began testing it as a possible food for shrimp. H. C. Bold, Department of Botany, University of Texas at Austin (personal communication) with corroboration by Richard Norris, Department of Botany, University of Washington, Seattle, Washington (personal communication), identified the genus as *Tetraselmis chuii*. *Tetraselmis* is alternately known as *Platymonas*, but, in our work we follow the classification of Butcher (1959) who claims that *Tetraselmis* outdates *Platymonas*.

The purpose of this paper is to describe the techniques used for mass culturing *Tetraselmis* at the Galveston Laboratory of the National Marine Fisheries Service.

### METHODS AND MATERIALS

Stock cultures of *Tetraselmis* are maintained in 16 x 125 mm screw-cap glass culture tubes. Cultures to produce inoculum are also started in these tubes and transferred later to 25 x 200 mm screw-cap glass culture tubes. The next container used is a 9-liter glass carboy, and the final culture container is a rectangular polyethylene tank of approximately 340-liter capacity.

All glassware is washed thoroughly and autoclaved. The plastic culture tanks are scrubbed and then rinsed with dilute nitric acid to remove the carbonate particles that form on the sides and bottom of the tank. The container is then rinsed once more, allowed to drain, and placed under an ultraviolet sterilizer (Griffith, 1971).

Stock cultures of *Tetraselmis* are maintained in sterilized artificial seawater medium referred to as "NH" or "NH 15" (Gates and Wilson, 1960). This is not a medium that promotes dense growth but one in which the cells are sustained in good condition. Maintenance tubes of *Tetraselmis* are sub-cultured at 4-day intervals to keep the alga reproducing actively. Salinity of the NH medium is 28 ppt and culture room temperature is 24 C  $\pm$  2. Cultures are kept under constant illumination about 5 cm from two 30-watt cool white fluorescent tubes, producing approximately 4,000 foot candles of light at the outside surface of the tubes.

Cultures used as inocula must be started approximately 16 days prior to the first harvest from the 340-liter tanks. At this time two 16 x 125 mm culture tubes containing NH medium are inoculated with maintenance stock. The cells reproduce for 4 days in the two small tubes, and are then dispensed equally into three 25 x 200 mm tubes of NH medium. After 4 days the three large culture tubes are emptied into a 9-liter glass carboy containing 2 liters of enriched artificial seawater.

The algal cells are kept in suspension by a magnetic stir

bar and stirring motor. Then on successive days, the volume in the carboy is doubled by adding first 2 liters then 4 liters of enriched artificial seawater. Nothing is added to the carboy on the 4th day. On the morning of the 5th day, 96 hours after inoculation of the carboy, it is ready to use as inoculum for a large culture tank.

Then enriched artificial seawater used for the large cultures is made with Instant Ocean<sup>2</sup> and tap water (salinity 28 ppt) plus the following additives per 100 liters: potassium nitrate, 10 g; EDTA (ethylene-dinitrilo tetracetic acid di-sodium salt), 1 g; ferric ammonium sulfate, 1 g; and 400 ml Tris buffer (2-amino-2-hydroxy-methyl-1, 2 propanediol) adjusted to pH 8.3 with concentrated hydrochloric acid.

Similar to the carboy method above, the large cultures are ready for harvest on the morning of the fifth day. Preceding the day the culture is started, artificial seawater is prepared in a large reservoir container by mixing Instant Ocean and tap water to 28 ppt salinity. The algae are cultured as follows in a 340-liter tank:

| Day of culture | Amount of Instant Ocean added | Amount of KNO <sub>3</sub> , EDTA, FeNH <sub>4</sub> (SO <sub>4</sub> ) <sub>2</sub> added | Amount of Tris buffer added | Inoculation w/ prepared Tetraselmis | Harvest        |
|----------------|-------------------------------|--------------------------------------------------------------------------------------------|-----------------------------|-------------------------------------|----------------|
| 1st day        | 100 liters                    | 10 g KNO <sub>3</sub> , 1 g EDTA<br>1 g FeNH <sub>4</sub> (SO <sub>4</sub> ) <sub>2</sub>  | 400 ml                      | 8 liters                            | None           |
| 2nd day        | 100 liters                    | 20 g KNO <sub>3</sub> , 1 g EDTA<br>2 g FeNH <sub>4</sub> (SO <sub>4</sub> ) <sub>2</sub>  | 400 ml                      | None                                | None           |
| 3rd day        | 100 liters                    | 30 g KNO <sub>3</sub> , 3 g EDTA<br>3 g FeNH <sub>4</sub> (SO <sub>4</sub> ) <sub>2</sub>  | 400 ml                      | None                                | None           |
| 4th day        | None                          | None                                                                                       | None                        | None                                | None           |
| 5th day        | None                          | None                                                                                       | None                        | None                                | All of culture |

Aeration and circulation of cultures in the 340-liter tanks are accomplished using the circulator described by Salser (Ms). Compressed air is used for aeration. The circulating device provides for continuous turnover of all the water in the tank, exposing all cells to the light source, which consists of a series of 10 cool white 86-watt, 183-cm flourescent tubes suspended approximately 15 cm above the surface of the water, producing about 32,292 lux of light at the water surface.

<sup>2</sup>The use of trade names in this publication does not imply endorsement of commercial products.

The temperature is maintained at  $21 \pm 2$  C during mass culture of *Tetraselmis*. Cell counts of the cultures are made with a hemacytometer. After cell counts are made, the cultures are harvested by centrifuging the cells out of the culture medium with a De Laval Model 108 milk separator.<sup>3</sup> Zein-Eldin (Galveston Laboratory, personal communication) has observed that spent algal medium is toxic to postlarval shrimp. The concentrated algal cells are either used as food immediately or can be preserved for future use by freezing or by freeze-drying (Brown, 1972).

## RESULTS AND DISCUSSIONS

Typical densities produced in the mass-culture tanks with these methods range from 275,000 to 450,000 cells per ml. These cultures are maintained in the log-phase of growth to standardize the quality of the cells. We have produced cultures with as many as 1,500,000 cells per ml by continuing the culture more than 4 days.

*Tetraselmis* is an adaptable culture organism. It will thrive in outside tanks with natural light or under laboratory conditions with various light sources. *Tetraselmis maculata* was maintained in darkness at 20 C for 24 weeks and subsequently resumed growth (Antia and Cheng, 1970). Yanase and Imai (1968) tested light intensity for the related *Platymonas* sp. and found optimum light to be from 4,500 to 8,000 lux. They obtained maximum cell concentrations at 23 to 25 C. In our work, outdoor mass cultures have grown at temperatures from 15 to 35 C with only natural light.

*Tetraselmis* has produced cultures dependably in natural and artificial seawater medium with up to 1,000,000 cells per ml. We have had suitable cultures in natural seawater ranging in salinity from 15 ppt to 36 ppt and in artificial seawater from 22 ppt to 36 ppt. Sixty percent of the volume of outdoor seawater cultures, fertilized with nitrate, EDTA, and iron can be harvested daily. Under these conditions cultures continue to grow as long as 4 weeks. Contamination with other organisms can occasionally necessitate discontinuing a culture. *Tetraselmis* cultures have sometimes been contaminated with a phagotrophic dinoflagellate tentatively identified as *Oxyrrhis* sp. This can completely decimate a culture in 24 hours.

The volume of the culture of *Tetraselmis* does not appear to affect the final number of cells per ml. We have had peak numbers of cells in cultures from 10 ml in tubes to at least 10,000 liters in shrimp culture tanks. Culture medium may be gently stirred with air in some large cultures or vigorously agitated as in our mass culture techniques with no apparent harmful effect on the cells.

<sup>3</sup>The use of trade names in this publication does not imply endorsement of commercial products.

Experiments designed to stimulate cell division of Tetraselmis have been conducted in our laboratory. Thiamine-dihydrochloride, cobalamine, calcium-d-pantothenic acid, riboflavin, B-indole-acetic acid, nicotinic acid amide, and gibberellic acid have been added to cultures of the alga in artificial seawater medium. All additions were without effect except nicotinic acid amide which was detrimental.

Tetraselmis has been used as a food for several larval animals. Walne (1970) reported Tetraselmis sp. including T. chuii as an outstanding food for the juvenile bivalves Crassostrea ostrea, Mercenaria sp. and Mytilus sp. Biologists for the State of Texas are using the flagellate as satisfactory food for larval red drum, Sciaenops ocellata. Nimura (1967) found that brine shrimp, Artemia, need food with chlorophyll to provide the hemoglobin necessary for formation of viable resting eggs. Apparently Tetraselmis provides suitable nutrients since we have grown Artemia through all of its stages with this alga as the only food source. We have also cultured rotifers, Brachionus plicatilis using Tetraselmis as the only food. Development of larvae of Penaeus setiferus and P. aztecus is normal, and growth of the Protozoa II instar has been more rapid with Tetraselmis as the food source than with Skeletonema (C. R. Mock, Galveston Laboratory, personal communication).

## LITERATURE CITED

- Antia, N. J. and J. Y. Cheng. 1970. The survival of axenic cultures of marine planktonic algae from prolonged exposure to darkness at 20 C. *Phycologia* 9(2):179-183.
- Brown, Ausbon, Jr. 1972. Experimental techniques for preserving diatoms used as food for larval Penaeus aztecus. Proceedings of the National Shellfisheries Association 62:21-25.
- Butcher, R. W. 1959. An introductory account of the smaller algae of British coastal waters. Part 1: Introduction and Chlorophyceae Fishery Investigations Series IV. Ministry of Agriculture, Fisheries and Food. London: Her Majesty's Stationery Office.
- Cook, H. L., and M. A. Murphy. 1966. Rearing penaeid shrimp from eggs to postlarvae. Proceedings 19th Annual Conference Southeastern Association Game and Fish Commissioners 19: 283-288.
- Gates, J. A. and W. B. Wilson. 1960. The toxicity of Gonyaulax monilata Howell to Mugil cephalus. *Limnology and Oceanography* 5:171-174.
- Griffith, George W. 1971. Ultraviolet sterilization for algal culture. *FAO Fish Culture Bulletin* 4(1):7.

- Hudinaga, M. 1942. Reproduction, development, and rearing of Penaeus japonicus Bate. *Japanese Journal of Zoology* 10(2): 305-393.
- Mock, C. R., and M. A. Murphy. 1970. Techniques for raising penaeid shrimp from the egg to postlarvae. Proceedings 1st Annual Workshop World Mariculture Society 1:143-158.
- Nimura, Yoshihachiro. 1967. Biology of the brine shrimp. *Bulletin of the Japanese Society of Scientific Fisheries* 13(7):690-702.
- Salser, B. R. 1973. An air-lift circulator for algal culture. Proceedings 4th Annual Workshop World Mariculture Society (In press).
- Walne, P. R. 1970. Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera Ostrea, Crassostrea, Mercenaria and Mytilus. *Fishery Investigations, Series II, Vol. XXXI, No. 5*, Ministry of Agriculture, Fisheries and Food, Her Majesty's Stationery Office, London.
- Yanase, Ryosuki, and Takeo Imai. 1968. The effect of light intensity and temperature on the growth of several marine algae useful for rearing molluscan larvae. *Tohoku Journal Agriculture Research* 19(1):75-82.